

UTILITY PATENT APPLICATION TRANSMITTAL (Large Entity)

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Docket No.
1118/174

Total Pages in this Submission
254

TO THE UNITED STATES PATENT AND TRADEMARK OFFICE

Box Patent Application
Washington, D.C. 20231

Transmitted herewith for filing under 35 U.S.C. 111(a) and 37 C.F.R. 1.53(b) is a new utility patent application for an invention entitled:

METHOD FOR PERFORMING MICROASSAYS

and invented by:

IAN W. HUNTER

If a **CONTINUATION APPLICATION**, check appropriate box and supply the requisite information:

Continuation Divisional Continuation-in-part (CIP) of prior application No.: 09/225,583

Which is a:

Continuation Divisional Continuation-in-part (CIP) of prior application No.: _____

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Continuation Divisional Continuation-in-part (CIP) of prior application No.: _____

Enclosed are:

Application Elements

- Filing fee as calculated and transmitted as described below
- 2. Specification having 17 pages and including the following:
 - a. Descriptive Title of the Invention
 - b. Cross References to Related Applications (*if applicable*)
 - c. Statement Regarding Federally-sponsored Research/Development (*if applicable*)
 - d. Reference to Microfiche Appendix (*if applicable*)
 - e. Background of the Invention
 - f. Brief Summary of the Invention
 - g. Brief Description of the Drawings (*if drawings filed*)
 - h. Detailed Description
 - i. Claim(s) as Classified Below
 - j. Abstract of the Disclosure

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Application Elements (Continued)

3. Drawing(s) (*when necessary as prescribed by 35 USC 113*)
a. Formal Number of Sheets 9
b. Informal Number of Sheets _____
4. Oath or Declaration
a. Newly executed (*original or copy*) Unexecuted
b. Copy from a prior application (37 CFR 1.63(d)) (*for continuation/divisional application only*)
c. With Power of Attorney Without Power of Attorney
d. *DELETION OF INVENTOR(S)*
Signed statement attached deleting inventor(s) named in the prior application,
see 37 C.F.R. 1.63(d)(2) and 1.33(b).
- Incorporation By Reference (*usable if Box 4b is checked*)
The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under
Box 4b, is considered as being part of the disclosure of the accompanying application and is hereby
incorporated by reference therein.
- Computer Program in Microfiche (*Appendix*)
- Nucleotide and/or Amino Acid Sequence Submission (*if applicable, all must be included*)
a. Paper Copy
b. Computer Readable Copy (*identical to computer copy*)
c. Statement Verifying Identical Paper and Computer Readable Copy

Accompanying Application Parts

8. Assignment Papers (*cover sheet & document(s)*)
9. 37 CFR 3.73(B) Statement (*when there is an assignee*)
10. English Translation Document (*if applicable*)
11. Information Disclosure Statement/PTO-1449 Copies of IDS Citations
12. Preliminary Amendment
13. Acknowledgment postcard
14. Certificate of Mailing

First Class Express Mail (*Specify Label No.:* EL487322152US)

11-13-00

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**UTILITY PATENT APPLICATION TRANSMITTAL
(Large Entity)**

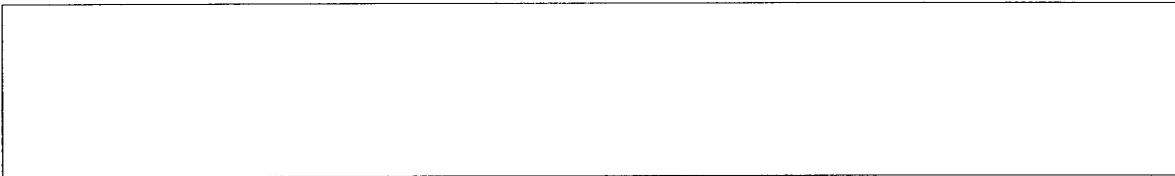
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Accompanying Application Parts (Continued)

15. Certified Copy of Priority Document(s) (*if foreign priority is claimed*)

16. Additional Enclosures (*please identify below*):


Fee Calculation and Transmittal

CLAIMS AS FILED

For	#Filed	#Allowed	#Extra	Rate	Fee
Total Claims	20	- 20 =	0	x \$18.00	\$0.00
Indep. Claims	4	- 3 =	1	x \$80.00	\$80.00
Multiple Dependent Claims (check if applicable)	<input type="checkbox"/>				\$0.00
				BASIC FEE	\$710.00
OTHER FEE (specify purpose)					\$0.00
				TOTAL FILING FEE	\$790.00

- A check in the amount of **\$790.00** to cover the filing fee is enclosed.
- The Commissioner is hereby authorized to charge and credit Deposit Account No. **19-4972** as described below. A duplicate copy of this sheet is enclosed.
- Charge the amount of _____ as filing fee.
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 - Charge any additional filing fees required under 37 C.F.R. 1.16 and 1.17.
 - Charge the issue fee set in 37 C.F.R. 1.18 at the mailing of the Notice of Allowance, pursuant to 37 C.F.R. 1.311(b).

Dated: **November 10, 2000**

cc:


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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor: Hunter

Atty Docket: 1118/174

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Art Unit: N/A

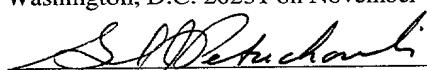
Date Filed: November 10, 2000

Examiner: N/A

For: METHOD FOR PERFORMING MICROASSAYS (as amended)

CERTIFICATE OF MAILING

I hereby certify that this document, along with any other papers referred to as being attached or enclosed, is being deposited with the United States Postal Service as express mail with sufficient postage in an envelope addressed to: Commissioner for Patents, Washington, D.C. 20231 on November 10, 2000.


Samuel J. Retuchowski

Commissioner for Patents
Washington, DC 20231

PRELIMINARY AMENDMENT
DIVISIONAL PATENT APPLICATION

Dear Sir:

Please amend the enclosed application as follows:

In the Specification

Before the first line of the application, please add the following language:

- This application is a divisional application of copending application U.S. Serial No. 09/225,583, filed January 5, 1999, which application is herein incorporated by reference. —

In the Title

Please delete the words "and Apparatus" from the title.

In the Abstract

Please replace the Abstract with the language provided on the separate page appended hereto in accordance with the requirements of 37 CFR §1.72.

In the Claims

Please cancel claims 2, 18-40.

Please amend claims 1, 5-7, 11, 12, and 14-16 and add new claim 41-44 to read as follows:

1. (amended) A method for [selecting samples having] analyzing specified properties [from] of a [library] set of samples, the method comprising:

- a. providing a platen having two substantially parallel planar surfaces and [a] a two-dimensional array [plurality] of addressable through-holes having at least 30 holes in each of two directions, the through-holes being disposed substantially perpendicularly to the planar surfaces;
- b. loading a first sample [in liquid form] into at least one of the through-holes;
- c. retaining the sample in the at least one of the through-holes by surface tension;
- d. adding a second sample into the at least one of the through-holes for permitting a reaction between the first sample and the second sample; and
- e. characterizing the reaction in the through-hole in terms of the specified properties.

5. (amended) A method according to claim [1] 43, wherein the first sample in liquid form includes at least one of a target in solution and a target in suspension.

6. (amended) A method according to claim [2] 43, wherein at least one of a target in solution and a target in suspension includes a biological material.

7. (amended) A method according to claim [1] 43, wherein the step of loading a first sample includes drawing the sample from a planar surface by capillary action.

11. (amended) A method according to claim [1] 43, further including maintaining a humid atmosphere for preventing evaporation of the first sample.

12. (amended) A method according to claim [1] 43, further including coating the liquid sample with a monolayer for preventing evaporation of the first sample.

14. (amended) A method for characterizing a plurality of [liquid] samples, the method comprising:

- a. providing a platen having a [set] two-dimensional array of through-holes having at least 30 holes in each of two directions;
- b. loading a specified [liquid] sample into each through-hole of a subset of the set of through-holes; and
- c. characterizing a property of the specified [liquid] sample.

15. (amended) A method according to claim 14, the step of characterizing a property of the specified [liquid] sample comprising:

- a. illuminating at least one through-hole of the subset of the set of through-holes with optical radiation; and
- b. analyzing the optical radiation emanating from the at least one through-hole.

16. (amended) A method for analyzing a plurality of [liquid] samples, the system comprising:

- a. loading the [liquid] samples into a plurality of through-holes disposed in a platen;
- b. illuminating at least one through-hole with optical radiation; and

analyzing the optical radiation emanating from the at least one through-hole.

—41. (new) A method for characterizing a plurality of samples, the method comprising:

- d. providing a platen having a two-dimensional array of through-holes;
- e. loading a specified sample into each through-hole of a subset of the set of through-holes with a density of at least one through-hole per square millimeter; and

characterizing a property of the specified sample.

—42. (new) A method according claim 1, wherein at least one of the first sample and second sample is in liquid form.

—43. (new) A method according claim 1, wherein the first sample is in liquid form.

—44. (new) A method according claim 14, 16, or 41, wherein at least one of the samples is in liquid form. —

REMARKS

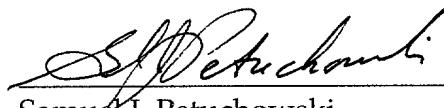
Claims 1, 5-7, 11, 12, and 14-16 are amended and claims 41-44 are added herein to more clearly recite the invention.

No new matter is included in the amended claims, and support for the amended language appears in the Specification as follows:

The limitation requiring the step of providing a platen having an array of at least 30 holes in each direction is supported in various places in the specification. For example, at p. 6, lines 13-19, the Specification teaches preferred hole dimensions (of 100-400 μm) and inter-hole spacing (on the order of twice the diameter of the holes). Based on this description, there is at least one hole per linear millimeter, since the inter-hole spacing is $\sim 800 \cdot \text{m}$. A preferred polymer disk dimension, described at page 6, line 27-29, is 100 mm, thus a total hole number of even greater than 100 holes along any radius is taught. In particular, the radii may be in various directions, as required by the claim. In fact, substantially higher densities (10^8 m^{-2} , or 100 per square millimeter) are described at p. 2, lines 12-13.

Examination and allowance of the claims as amended is respectfully solicited.

Respectfully submitted,



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134952



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PATENT TRADEMARK OFFICE

Abstract

A method for analyzing a plurality of samples. In accordance with the method, a platen is provided having two substantially parallel planar surfaces and a plurality of through-holes dimensioned so as to maintain a sample in each through-hole by means of surface tension. A source of optical radiation illuminates the through-holes, and an optical arrangement analyzes the light emanating from the through-holes. The through-holes may be individually addressable, and may have volumes less than 100 nanoliters. Samples may be drawn from a planar surface by capillary action and may be accurately dispensed, diluted and mixed in accordance with embodiments of the invention.

134952

Attorney Docket: 1118/163

Method and Apparatus for Performing Microassays

The present application claims priority from U.S. provisional application number
5 60/071,179 filed January 12, 1998, which application is herein incorporated by reference.

Technical Field

The present invention pertains to an apparatus and method for manipulating,
transporting, and analyzing a large number of microscopic samples of a liquid or of materials
10 including cells currently or formerly in liquid suspension.

Background of the Invention

Chemistry on the micro-scale, involving the reaction and subsequent analysis of
quantities of reagents or analytes of order microliters or smaller, is an increasingly important
15 aspect of the development of new substances in the pharmaceutical and other industries. Such
reaction and analysis may accommodate vast libraries containing as many as a million
compounds to be reacted and analyzed under various conditions. Significant problems
associated with current technologies as applied to chemical analysis of vast numbers
(potentially on the order of hundreds of thousands or millions per day) of compounds include
20 the problem of handling vast numbers of compounds and reactions in parallel.

Existing technology relies on 96-, 384-, or 1536-well plates containing quantities
between approximately 1 microliter and 1 milliliter of liquid compound per well, and,
generally, involves chemical reactions and analysis in wells disposed with single openings on
flat, two-dimensional surfaces such as silicon chips. It is not practical to apply existing
25 technology in the art to form million-well disks. There is a need, therefore, for new
approaches that permit the analysis of a million samples in a laboratory format.

Summary of the Invention

In accordance with one aspect of the invention, in one of its embodiments, there is
30 provided a method for selecting samples having specified properties from a library of
samples. The method has the steps of:
a. providing a platen having two substantially parallel planar surfaces and a

plurality of addressable through-holes disposed substantially perpendicularly to the planar surfaces;

- b. loading a first sample in liquid form into at least one of the through-holes;
- c. adding a second sample into the at least one of the through-holes for permitting a reaction between the first sample and the second sample; and
- d. characterizing the reaction in the through-hole in terms of the specified properties.

In accordance with alternate embodiments of the invention, each through-hole may be dimensioned so as to maintain a liquid sample therein by means of surface tension, and may have a volume less than 100 nanoliters. The plurality of addressable through-holes may have a density in excess of 10^8 per square meter.

In accordance with further alternate embodiments of the invention, the step of loading a first sample may include drawing the sample from a planar surface by capillary action. The platen may be brought into contact with a reservoir of liquid and rotated about an axis perpendicular to the surface of the reservoir or about at least one of an axis perpendicular to the surface of the reservoir and an axis parallel to the surface of the reservoir. The method may include the further step of maintaining a humid atmosphere for preventing evaporation of the first sample or coating the liquid sample with a monolayer for preventing evaporation of the first sample.

In accordance with a further aspect of the present invention, a method is provided for preparing a plurality of combinations of members of a first set of samples in liquid form with members of a second set of samples in liquid form, the method comprising:

- a. providing a first perforated platen having through-holes and a second perforated platen having through-holes;
- b. loading a first set of samples in liquid form into the through-holes of the first perforated platen;
- c. loading a second set of samples in liquid form into the through-holes of the second perforated platen;
- d. registering the through-holes of the first perforated platen with the through-holes of the second perforated platen; and
- e. combining the first set of samples with the second set of samples.

In accordance with yet further aspects of the present invention, there are provided

methods for mixing and diluting liquid samples. The methods have steps of loading one set of liquid samples into through-holes of a first platen and loading another set of liquid samples into through-holes of a second platen, and then disposing a surface of the first platen in contact with a surface of the second platen in such a way as to register at least one through-hole of the first platten with at least one of through-hole of the second platten for permitting mixing of the liquid samples of the respective sets.

In accordance with another aspect of the present invention, there is provided a system for analyzing a plurality of liquid samples. The system has a platen having two substantially parallel planar surfaces and a plurality of through-holes having apertures and walls, a source of optical radiation for illuminating at least one through-hole along an optical axis, and an optical arrangement for analyzing light emanating from the at least one through-hole.

Brief Description of the Drawings

The foregoing features of the invention will be more readily understood by reference 15 to the following detailed description taken with the accompanying drawings in which:

FIG. 1 is a side view in cross-section of a portion of a laminated platen containing multiple through-holes for analysis of liquid samples in accordance with a preferred embodiment of the present invention;

FIG. 2A is top view of a portion of the platen of FIG. 1 in which the through-holes are 20 configured on rectangular centers;

FIG. 2B is top view of a portion of the platen of FIG. 1 in which the through-holes are configured in a hexagonal close-packed array;

FIG. 3 is a top view of round sample wafer populated with through-holes in accordance with an embodiment of the present invention;

FIG. 4 is a side perspective view of an arrangement for loading a liquid sample into the platen of FIG. 1 by employing capillary and inertial insertion forces;

FIG. 5 is a cut-away view of a single through-hole in the platen of FIG. 1, showing the use of hydrophobic and hydrophilic layers for containment of an aqueous sample;

FIG. 6 is schematic diagram of a confocal optical arrangement for interrogation of a 30 liquid sample in a through-hole in accordance with an embodiment of the present invention;

FIG. 7 is perspective view of a scanning arrangement for serially interrogating liquid samples retained in through-holes of a disk-type platen in accordance with an embodiment of

the present invention;

FIG. 8 is schematic representation of a scanning arrangement for serially interrogating liquid samples retained in a continuous-process film-type platen, in accordance with an alternate embodiment of the present invention;

5 FIG. 9 is a cross-sectional view of portions of two platens brought into proximity with through-hole registration in anticipation of mixing or dilution in accordance with embodiments of the present invention; and

FIG. 10 is a cross-sectional view of the portions of two platens of FIG. 9 after the two platens have been brought into contact to facilitate mixing or dilution.

10

Detailed Description of Preferred Embodiments

Through-hole wells

In accordance with a preferred embodiment of the invention, the volume of each well employed for the assay of a chemical or biochemical reaction is reduced typically to less than

15 100 nanoliters (10^{-10} m³). The packing density of wells may thereby be increased by several orders of magnitude over prior art technology. Referring to FIG. 1, a side view is shown in cross section of a platen 10, otherwise referred to herein as a "substrate" or "sample wafer." Platen 10 is the carrier of a large number of through-holes 12 which traverse platen 10 from one surface 14 to an opposing surface 16 of the platen and constitute assay wells (or
20 "microwells") in accordance with an embodiment of the invention. Through-holes 12 may be shaped as circular right cylinders, or, alternatively, may have rectangular cross-sections, however otherwise shaped through-holes are within the scope of the present invention. As used in the present description and in the appended claims, the term "platen" refers to a structure having substantially parallel plane surfaces and transverse dimensions substantially
25 exceeding the thickness of the structure between the substantially parallel plane surfaces.

The apertures of through-holes 12 need not be square, and, in accordance with an alternate embodiment of the present invention, flanges 8 may extend above planar surface 14 surrounding some or all of through-holes 12 while indentations 6 may be fabricated rounding the edges of through-holes 12 at opposing surface 16. Flanges 8 and indentations 6 may
30 advantageously provide for registration of successive platens 10, in the case where platens are stacked, and in processes of mixing or dilution, as discussed in detail below in reference to

Figs. 9-10.

In accordance with an embodiment of the invention, through-holes **12** are loaded with a first sample **18** in liquid form. Sample **18** is allowed to react with a second sample where the second sample may include a variety of test samples and by subsequent or concurrent 5 analysis of the reaction products, using, for example, optical markers, a large number of reactions may be processed and analyzed in parallel.

As applied to biological assays, by way of example, first sample **18** may be a reagent, including, for example, cells in aqueous suspension, eukaryotic (animal, yeast) or prokaryotic (bacteria) cells, hybrid cells, and biological molecules including, for example, antibodies and 10 enzymes, although application to other biological or non-biological assays is within the scope of the invention as claimed herein. All such reagents may also be referred to herein and in the appended claims as "targets." Typical yeast cell concentrations of 10^7 cells per milliliter of solution yield on the order of 1000 cells per 100 nanoliter well. Typically, an entire chip or the subset of through-hole wells constituting a contiguous region of platen **10** may be 15 populated with a single strain of cells.

A typical procedure assay procedure, such as may be employed in pharmaceutical research, entails the subsequent addressed introduction of a test sample including one or more analytes into the through-hole wells, with selected materials introduced into subsets of through-holes that may include one or more through-holes. The test sample addressably 20 introduced into the subsets of through-holes may contain drug candidates or known drugs. The test sample may be comprised of multiple components, introduced at the same time or sequentially. Components of the test sample may include analytes, antagonists, reagents, solvents, or any other materials and may be introduced in liquid form or otherwise. In accordance with a preferred embodiment of the invention, test samples are introduced into the 25 through-hole wells in liquid form in order to facilitate rapid reaction via diffusion with first sample **18** already resident in liquid form in the through-holes.

The set of substances from which the second sample addressed to a particular through-hole site is drawn is referred to in this description and in the appended claims as a "library" of substances. In typical applications, the library is of a substantial size and thus 30 advantageously utilizes the capability of the present invention to facilitate parallel reaction and analysis of large numbers of substances. In pharmaceutical applications in particular,

libraries may be composed of between 10^3 and 10^9 substances and combinations of substances.

A typical thickness **20** of platen **10** is on the order of 1-2 mm, while through-holes **12** have typical characteristic dimensions (such as diameters) **22** of on the order of 100-400 μm .

- 5 Thus the volume of each through-hole **12** between surface **14** and surface **16** is on the order of $\sim 10^{-7} \text{ cm}^3$ or greater. Through-holes **12** are spaced on centers typically on the order of twice the diameter of the holes, although all spacing configurations are within the scope of the invention and of the appended claims. In particular, through-holes **12** may be centered on a rectangular grid, as shown in FIG. 2A, or in a close-packed hexagonal lattice, as shown in
10 FIG. 2B.

In accordance with an alternate embodiment of the present invention described with reference to FIG. 3, through-holes **12** may be disposed in an array within a circular sample wafer **300** having a central hole **302** for purposes of centering with respect to handling equipment.

- 15 Referring again to FIG. 1, platen **10** may be any solid or quasi-solid material into which through-holes **12** may be formed. In particular, in accordance with various embodiments of the invention, platen **10** may be formed from metal, semiconductor, glass, quartz, ceramic or polymer materials, all given without limitation by way of example. In accordance with a preferred embodiment of the invention, platen **10** is formed in a format
20 associated with a compact disk read-only-memory (CD-ROM) - namely that of a polymer disk, approximately 1.2 mm in thickness, and approximately 100 mm in diameter.

Platen **10** may also advantageously be formed of a laminate of materials, with a central layer **26** and outer "sandwiching" layers **28**. Advantages of this construction for containment of sample **18** will be discussed further below.

- 25 Through-holes **12** may be formed in platen **10** by means appropriate to the material of platen **10**. Through-hole forming methods include, by way of example, laser ablation by means of an ultraviolet (UV) excimer laser which may form 100 μm through-holes in glasses and polymers. Additional through-hole forming techniques include mechanical drilling, electrochemical methods, or selective chemical or charged-particle etching techniques.
30 Additionally, microcapillary bundles of glass fibers of varying compositions may be drawn from preform and sliced to form platens, and then selectively etched to form through-holes.

Loading the through-hole microwells

On the size scale employed in accordance with embodiments of the invention, where through-holes **12** have aspect ratios of axial length to diameter greater than unity, viscous forces may dominate inertial forces in governing the fluid kinetics of material in the through-

5 hole wells. Consequently, capillary action may be employed to populate through-holes **12** with sample fluid **18**. Referring to FIG. 4, two aspects of loading the through-hole wells are described with reference to a sample insertion apparatus **30**. Since through-hole microwells **12** are open at both sides, insertion of liquid into the wells does not require that the air displaced by the liquid on insertion flow through the entering fluid, as occurs in the prior art

10 well structure having only a single aperture for influx of liquid and efflux of displaced air.

Liquid **32**, loaded into reservoir **34** via port **33**, may, as discussed above, contain cells or other particles in suspension. Liquid **32** may be forced into through-hole microwells **12** (shown in FIG. 1) by in-line impulsion as by driving platen **10** into liquid **32** by force applied along direction **36** transverse to the plane of platen **10**. The transverse piston force may be

15 applied via shaft **38** or in any other manner known in the mechanical arts.

In accordance with another embodiment of the invention, liquid may also be loaded through capillary action of liquid **32** along the walls of the through-holes. To provide for wetting of the lower surface of platen **10**, the platen is lowered into reservoir **34** and rotated, by torque applied through shaft **38**, or otherwise, through an angle typically on the order of a

20 quarter revolution. Alternatively, platen **10** may be wetted and liquid **32** drawn into the microwells by immersing platen **10** into liquid **32** and tilting the platen about an axis in the plane of the platen.

Stabilization with respect to capillary and evaporative liquid loss

25 In order to maintain the sample in liquid form in the respective microwells, evaporation of the liquid must be avoided. One method of avoiding evaporation is to provide an ambient atmospheric environment of 100% humidity. Among other methods that may be practiced to suppress evaporation, in accordance with an embodiment of the invention, a high molecular-weight fluid, such as various alcohols, for example, may be introduced on each end

30 of the microwells thereby forming molecular monolayers or other thin layers to prevent evaporation of the liquid sample.

Referring to FIG. 5, a cross-section of a portion of platen **10** is shown to include through-hole microwell **12**. In order to enhance capillary loading of the microwell and to prevent capillary outmigration of the sample liquid, exterior sections **40** of the microwell, adjacent to surfaces **14** and **16** of platen **10**, has a hydrophobic wall surface in accordance
5 with a preferred embodiment of the invention, while the interior section **42** of the through-hole wall has a hydrophilic surface thereby preferentially attracting an aqueous liquid sample. Typically, the interior ~160 µm segment of the microwell may have a hydrophilic wall surface, while the hydrophobic layers on either end of the well are on the order of 20 µm in length. On loading the sample liquid into the microwells, typically 10% of the well, on either
10 end, is left unfilled, and subsequent test samples in liquid form will rapidly diffuse to hydrophilic center of microwell thereby mixing with the liquid already present.

Optical interrogation

Depending upon the application to which the present invention is applied, the result of
15 the reaction of the first sample in liquid form with subsequently added analytes may be read out in a wide variety of manners known to persons skilled in the biological or biochemical arts. Readout systems may employ taggants of various sorts allowing interrogation of the sample within the addressable microwell to determine whether a specified reaction has occurred. Some reactions may be interrogated optically, to include, without limitation, such
20 optical methods as colorimetric or fluorometric methods, or resonant or non-resonant scattering methods, including Raman spectroscopic methods.

Referring now to FIG. 6, optical interrogation methods, of which the foregoing are but examples, may be implemented, in accordance with an embodiment of the invention by coupling a light beam **50** into through-hole **12** of platen **10** and detecting light **52** emergent
25 from the opposite aperture of through-hole **12** by detectors **54** constituting detector array **56**. Alternatively, light returned by scattering in the original direction can be collected and analyzed using standard optical techniques. In order to optimize the signal-to-noise of the optical signal, the beam shape and through-hole volume are preferably matched. In
30 accordance with a preferred embodiment of the invention, optical matching to a through-hole of cylindrical cross-section and of aspect ratio greater than one is achieved through a confocal optical geometry in which an initially collimated beam **50** is transformed by optical element

- 58 into a beam having a diffraction limited focus at the center 60 of through-hole 12. The emergent optical beam 52 is collected and focussed onto detector array 56 by optical element 60. Superior optical sampling of the volume of the through-hole may be obtained if the through-hole has a rectangular cross-section, and if the optical radiation is guided by the
5 walls of the through-hole in the manner of a waveguide. Optical element 58 and 60 may be lenses or mirrors or combinations thereof as well known to persons skilled in the optical arts. Detector array 56 may be a charge-coupled device (CCD) array, for example, and, in one embodiment of the invention, a 1000×1000 element format is employed, with each through-hole imaged onto three elements 54 of the detector array. A window 62 may be disposed
10 between platen 10 and detector array 56 and may be dried using standard techniques if the assay is conducted in a humid ambient environment as discussed above. Alternatively, beam 50, coupled into through-hole 12 by coupling element 58 may be guided, in the manner of a guided wave through a waveguide, by the walls 62 of through-hole 12 in order to provide efficient interrogation of the sampled volume within the through-hole.
15 In some cases, where the material of platen 10 is not entirely opaque at the wavelengths of interrogating optical beam 50, wall 62 of through-hole 12 may be coated to prevent light leakage and cross-talk among the addressable sample volumes.

- FIG. 7 shows a preferred embodiment of the present invention in which platen 10 is configured in the CD-ROM format described above, with interrogating optical source 50
20 capable of travel in radial direction 68 while platen 10 rotates about center 66. Optical detector array 56 may translate in conjunction with source 50, in accordance with an embodiment of the invention.

Continuous process analysis

- 25 Referring to FIG. 8, in accordance with an advantageous embodiment of the present invention, platen 10, which may be a flexible polymeric substance, for example, is conveyed in a direction 70 past an optical interrogation system comprising an optical source 72 and a detector array 74. Samples in liquid form may be loaded into through-holes 12 and advanced at a rate governed by the relevant reaction times so that a row 76 is interrogated optically at
30 the period during which a specified indication is expected.

Mixing and Dilution

Referring now to Fig. 9, a cross-sectional view is shown of portions of a first platen 90 and a second platen 92 brought into proximity with each other in anticipation of processes performed in accordance with embodiments of the present invention for preparing, mixing, or diluting liquid samples. Through-holes 12 of platen 90 are shown as having been loaded with liquid samples 94 which may be identical across some specified subset of through-holes 12, or may be identical for the entire platen. Liquid sample 94, as shown schematically, may include cells or other targets 96 in solution within a solvent 98.

Through-holes 12 of second platen 92 is shown as having been loaded with liquid samples 100 and 102 shown comprising one or more solvents or other agents. In particular, platen 92 may have been populated with a library of distinct compounds, each of which is to be exposed to target 96 of platen 90.

Fig. 10 shows platens 90 and 92 of Fig. 9 having been brought into contact with one another, in such a manner as to allow through-holes of the respective platens to register on a one-to-one basis. The mating of protrusions 8 with indentations 6 of respective platens facilitates the registration of through-holes, and provides for the mixing of the liquid sample contents of the respective through-holes. Thus, as shown, half of targets 96 from samples 94 of first platen 90 have migrated to the solvent of samples 100 and 102. Mixing or dilution may be facilitated in this manner, either through ordinary statistical diffusion, or by any method employed to facilitate mixing. Mixing may be enhanced, for example, by the creation of thermal eddy currents and turbulence induced by laser irradiation. Mixing rates have been found to be enhanced in this way by more than an order of magnitude. Any other mixing techniques, including acoustic perturbation or stirring of the samples with micropipettes, for example, are within the scope of the present invention as described herein and as claimed in any appended claims.

The number of platens 90 and 92 that may be stacked, in accordance with the present invention, is not limited to two, as shown in Figs. 9 and 10 by way of example only. Thus, the concentration of targets 96 in solvent 98 may be diluted to a specified degree by stacking a corresponding number of platens with registered through-holes and allowing migration of targets 96 throughout the liquid contained within the corresponding sample volumes of the stack.

Transportation of biological samples

- The perforated platen described herein in accordance an embodiment of the present invention may be employed, for example, for shipping samples of a uniform strain of cells to 5 laboratories. In this application, the cells or other biological sample may be introduced into the through-hole wells of the invention in aqueous or other liquid suspension. The liquid carrier is then evaporated, allowing the cells or other biological samples to form a coating, in the form of a chimney, of the walls of the plurality of through-hole wells. The samples may then subsequently be resuspended by wetting and further analytes may be introduced.
- 10 The described embodiments of the invention are intended to be merely exemplary and numerous variations and modifications will be apparent to those skilled in the art. All such variations and modifications are intended to be within the scope of the present invention as defined in the appended claims.

I CLAIM:

1. A method for selecting samples having specified properties from a library of samples, the method comprising:

- a. providing a platen having two substantially parallel planar surfaces and a plurality of addressable through-holes disposed substantially perpendicularly to the planar surfaces;
- b. loading a first sample in liquid form into at least one of the through-holes;
- c. adding a second sample into the at least one of the through-holes for permitting a reaction between the first sample and the second sample; and
- d. characterizing the reaction in the through-hole in terms of the specified properties.

5 2. A method according to claim 1, wherein each through-hole is dimensioned so as to maintain a liquid sample therein by means of surface tension.

10 3. A method according to claim 1, wherein each through-hole has a volume less than 100 nanoliters.

15 4. A method according to claim 1, wherein the plurality of addressable through-holes has a density in excess of 10^8 per square meter.

5 5. A method according to claim 1, wherein the first sample in liquid form includes at least one of a target in solution and a target in suspension.

20 6. A method according to claim 2, wherein the at least one of a target in solution and a target in suspension includes a biological material.

7. A method according to claim 1, wherein the step of loading a first sample includes drawing the sample from a planar surface by capillary action.

25 8. A method according to claim 1, wherein the step of loading a first sample includes bringing the platen into contact with a reservoir of liquid and rotating the platen about an axis perpendicular to the surface of the reservoir.

9. A method according to claim 1, wherein the step of loading a first sample includes bringing the platen into contact with a reservoir of liquid and rotating the platen about at least one of an axis perpendicular to the surface of the reservoir and an axis parallel to the surface of the reservoir.

30 10. A method according to claim 1, wherein the step of loading a first sample includes

- bringing the platen into contact with a reservoir of liquid and impelling the platen in a direction substantially perpendicular to the planar surfaces of the platen.
11. A method according to claim 1, further including maintaining a humid atmosphere for preventing evaporation of the first sample.
- 5 12. A method according to claim 1, further including coating the liquid sample with a monolayer for preventing evaporation of the first sample.
13. A method according to claim 1, wherein the step of characterizing the reaction in the through-hole in terms of the specified properties includes optically analyzing the sample.
- 10 14. A method for characterizing a plurality of liquid samples, the method comprising:
- providing a platen having a set of through-holes;
 - loading a specified liquid sample into each of a subset of the set of through-holes; and
 - characterizing a property of the specified liquid sample.
- 15 15. A method according to claim 14, the step of characterizing a property of the specified liquid sample comprising:
- illuminating at least one through-hole of the subset of the set of through-holes with optical radiation; and
 - analyzing the optical radiation emanating from the at least one through-hole.
- 20 16. A method for analyzing a plurality of liquid samples, the system comprising:
- loading the liquid samples into a plurality of through-holes disposed in a platen;
 - illuminating at least one through-hole with optical radiation; and
 - analyzing the optical radiation emanating from the at least one through-hole.
- 25 17. A method in accordance with claim 16, wherein the step of analyzing includes spectrally characterizing the optical radiation emanating from the at least one through-hole.
18. A method for preparing a plurality of combinations of members of a first set of samples in liquid form with members of a second set of samples in liquid form, the method comprising:
- providing a first perforated platen having through-holes and a second

- perforated platen having through-holes;
- 5 b. loading a first set of samples in liquid form into the through-holes of the first perforated platen;
- c. loading a second set of samples in liquid form into the through-holes of the second perforated platen;
- d. registering the through-holes of the first perforated platen with the through-holes of the second perforated platen; and
- e. combining the first set of samples with the second set of samples.
- 10 19. A method according to claim 18, wherein the step of combining includes inertially injecting the first set of samples into the through-holes of the second perforated platen.
- 15 20. A method for mixing a plurality of liquid samples, the method comprising:
- a. loading a first set of liquid samples into a plurality of through-holes disposed in a first platen, the platen having a first substantially planar surface and a second substantially planar surface, the surfaces being substantially parallel to each other;
- b. loading a second set of liquid samples into a plurality of through-holes disposed in a second platen, the platen having a first substantially planar surface and a second substantially planar surface, the surfaces being substantially parallel to each other;
- c. disposing the first planar surface of the first platen in contact with the first planar surface of the second planar surface in such a manner that at least one through-hole of the first set is in registration with at least one through-hole of the second first set.
- 20 21. A method according to claim 20, wherein the first set of liquid samples includes a solute dissolved in a solvent and the second set of liquid samples includes a solvent, such that the concentration of solute in the first set of liquid samples is diluted upon performance of the step of disposing.
- 25 22. A method according to claim 20, further comprising:
- 30 d. mixing a liquid of the first set of liquid samples with a liquid of the second set of liquid samples.

23. A method according to claim 22, wherein the step of mixing includes inducing turbulence within the liquid by optical means.
24. A method according to claim 22, wherein the step of mixing includes inducing turbulence within the liquid by acoustic means.
- 5 25. A method according to claim 22, wherein the step of mixing includes inducing turbulence within the liquid by mechanical means.
26. A method for transporting biological samples, the method comprising:
- 10 a. providing a platen having a set of substantially cylindrical through-holes having walls;
- b. loading the biological samples suspended in a liquid carrier into the through-holes; and
- c. evaporating the liquid carrier for causing the biological samples to deposit on the walls of the through-holes.
27. A perforated platen having substantially parallel planar surfaces for manipulating liquid samples, the platen comprising:
- 15 a. an inner layer of hydrophilic material;
- b. two outer layers of hydrophobic material coupled to opposite sides of the inner layer; and
- c. through-holes for retaining the liquid samples, the through-holes traversing the inner layer and the two outer layers in a direction substantially perpendicular to the planar surfaces of the platen.
- 20 28. A system for analyzing a plurality of liquid samples, the system comprising:
- a. a platen having two substantially parallel planar surfaces and a plurality of through-holes having apertures and walls;
- 25 b. a source of optical radiation for illuminating at least one through-hole along an optical axis; and
- c. an optical arrangement for analyzing light emanating from the at least one through-hole.
29. A system according to claim 28, wherein the apertures of the plurality of through-holes are disposed on centers of a hexagonally close-packed lattice on the surface of the platen.
- 30

30. A system according to claim 28, wherein the apertures of the plurality of through-holes are disposed on centers of a rectangular lattice on the surface of the platen.
31. A system according to claim 28, wherein the through-holes have an aspect ratio of axial to transverse dimension of greater than 1.5.
- 5 32. A system according to claim 28, wherein the volume enclosed by the wall of each through-hole and the planes of the planar surfaces of the platen is less than 100 nanoliters.
33. A system according to claim 28, wherein the wall of each through-hole is in part hydrophilic and in part hydrophobic.
- 10 34. A system according to claim 28, wherein the wall of each through-hole comprises:
- a. a central hydrophilic segment; and
 - b. two hydrophobic segments such that one hydrophobic segment extends from the central hydrophilic segment to each planar surface of the platen.
35. A system according to claim 28, wherein the platen is a laminate having a central hydrophilic layer and two outer hydrophobic layers disposed on opposite sides of the central hydrophilic layer.
- 15 36. A system according to claim 28, wherein the platen is a metal.
37. A system according to claim 28, wherein the platen is a material selected from the group consisting of amorphous materials, ceramic, glass, quartz, and glassy carbon.
- 20 38. A system according to claim 28, wherein the platen is a polymeric material.
39. A system according to claim 28, wherein the walls of the plurality of through-holes are coated for allowing emission of light from the through-holes only at the planar surfaces of the platen.
40. A system according to claim 28, further including an advancement mechanism for translating the platen in a direction perpendicular to the optical axis.
- 25

Abstract

A system and method for analyzing a plurality of liquid samples. The system has a platen having two substantially parallel planar surfaces and a plurality of through-holes dimensioned so as to maintain a liquid sample in each through-hole by means of surface tension. A source of optical radiation illuminates the through-holes, and an optical arrangement analyzes the light emanating from the through-holes. The through-holes may be individually addressable, and may have volumes less than 100 nanoliters. Samples may be drawn from a planar surface by capillary action and may be accurately dispensed, diluted and mixed in accordance with embodiments of the invention.

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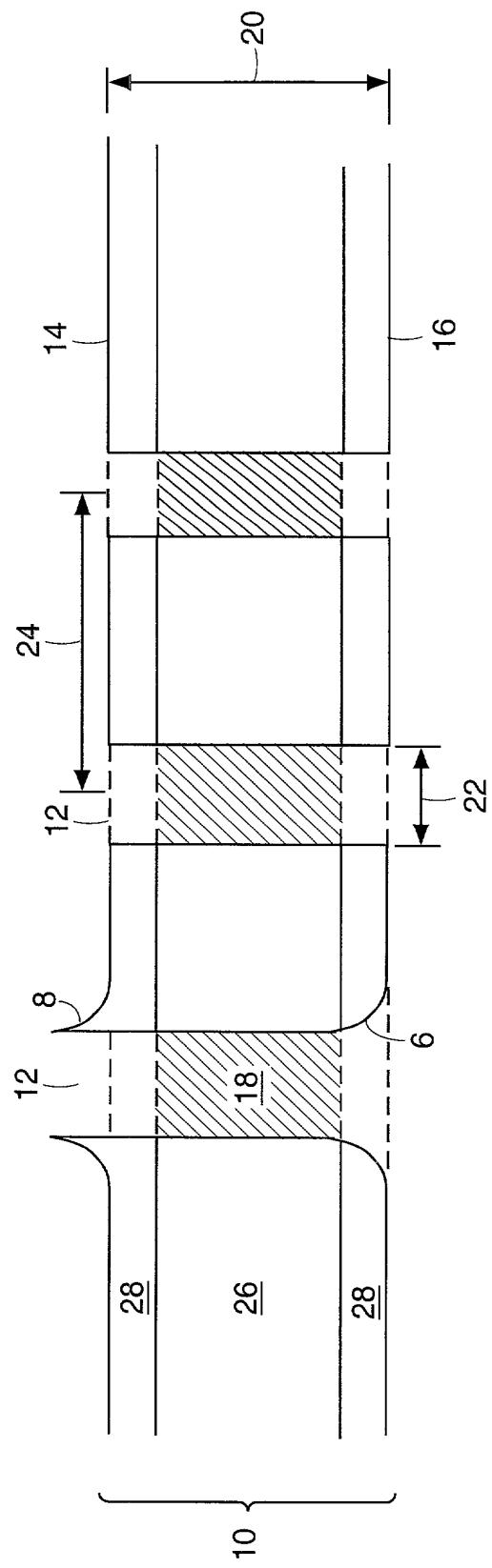
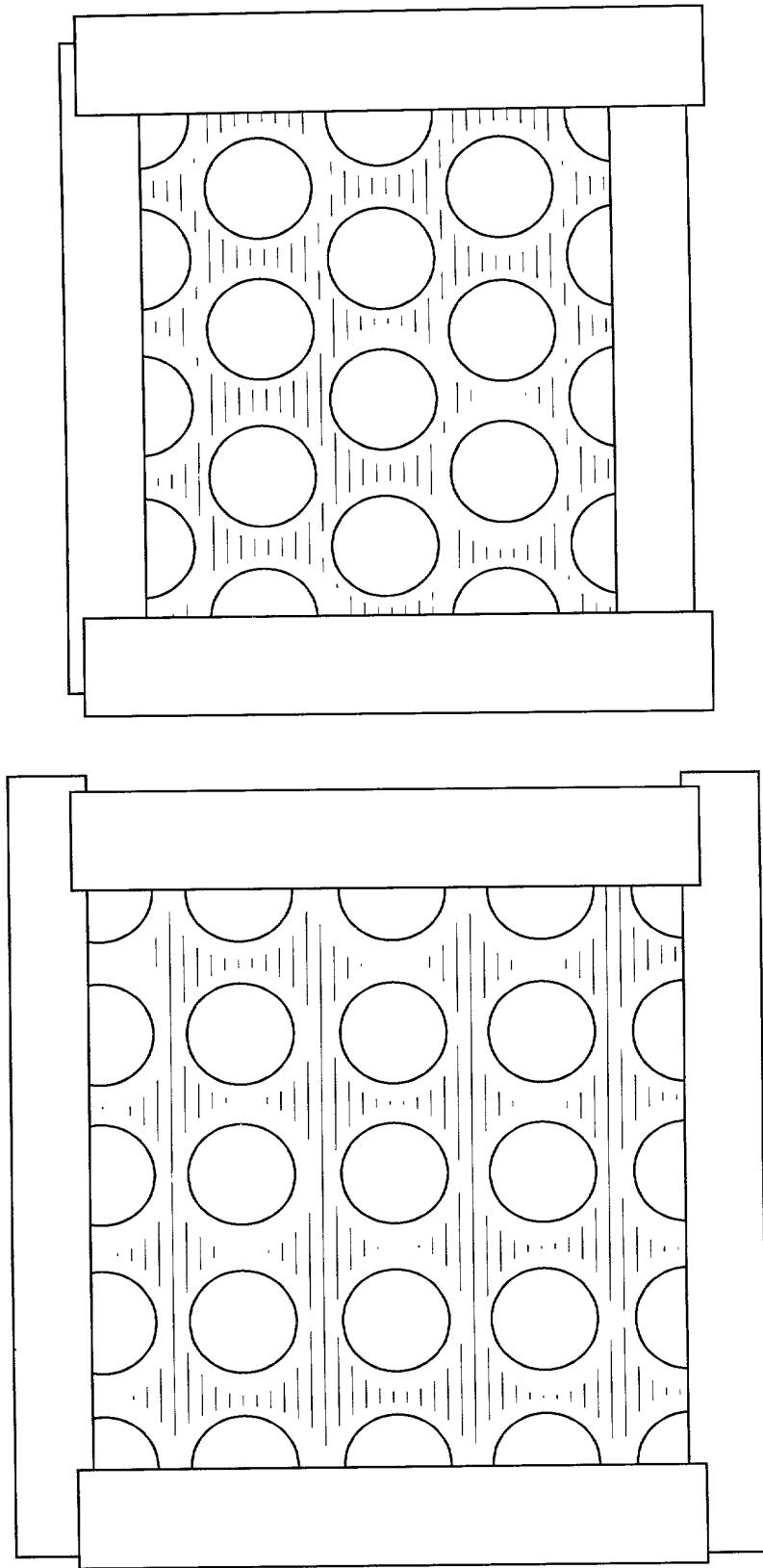


FIG. 1



RECTANGULAR PACKING

CLOSE PACKING

FIG. 2A

FIG. 2B

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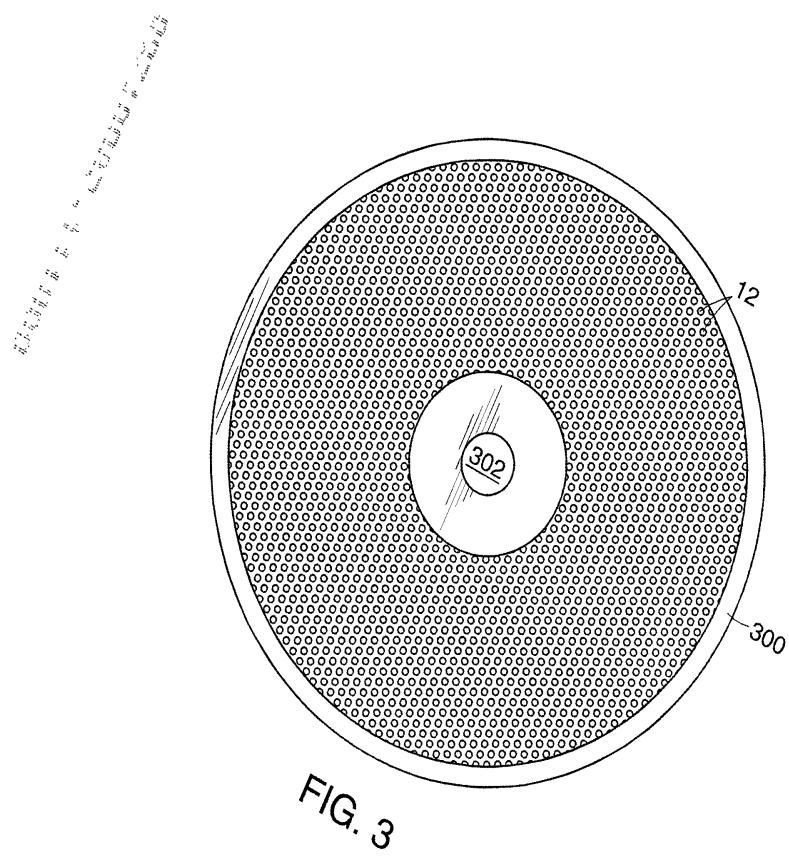


FIG. 3

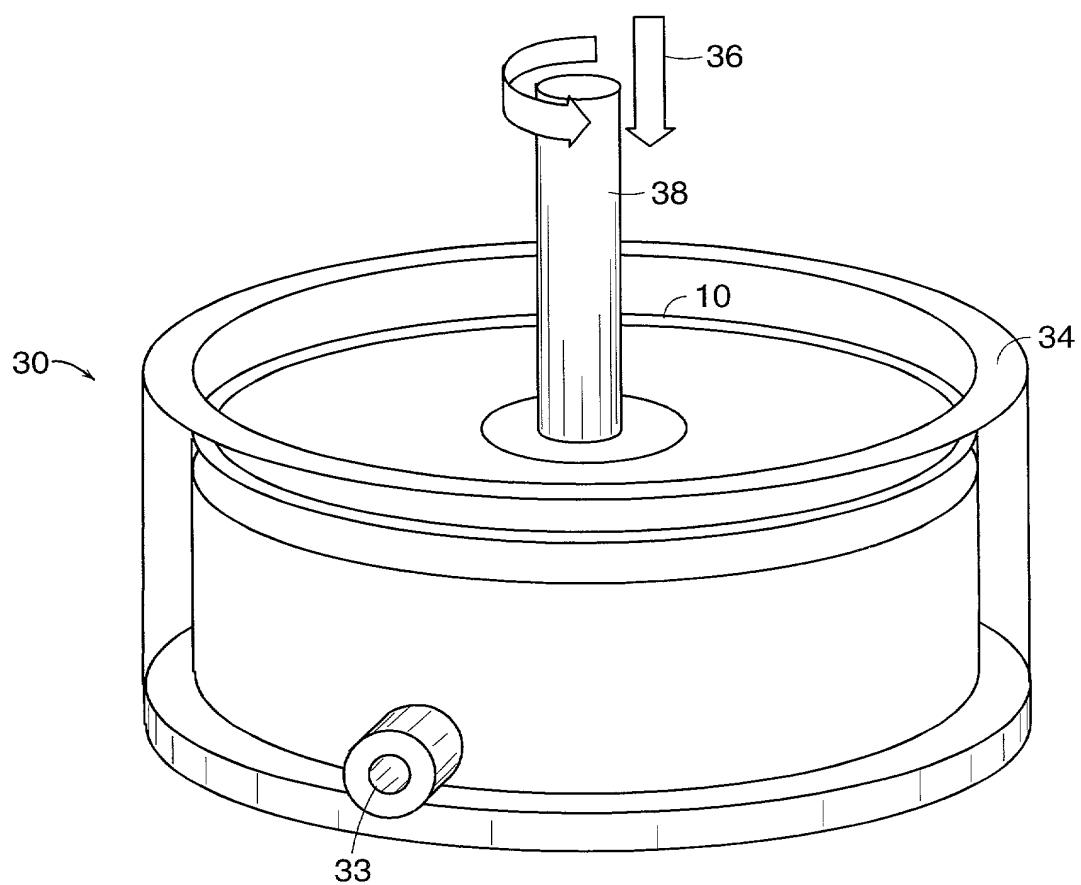


FIG. 4

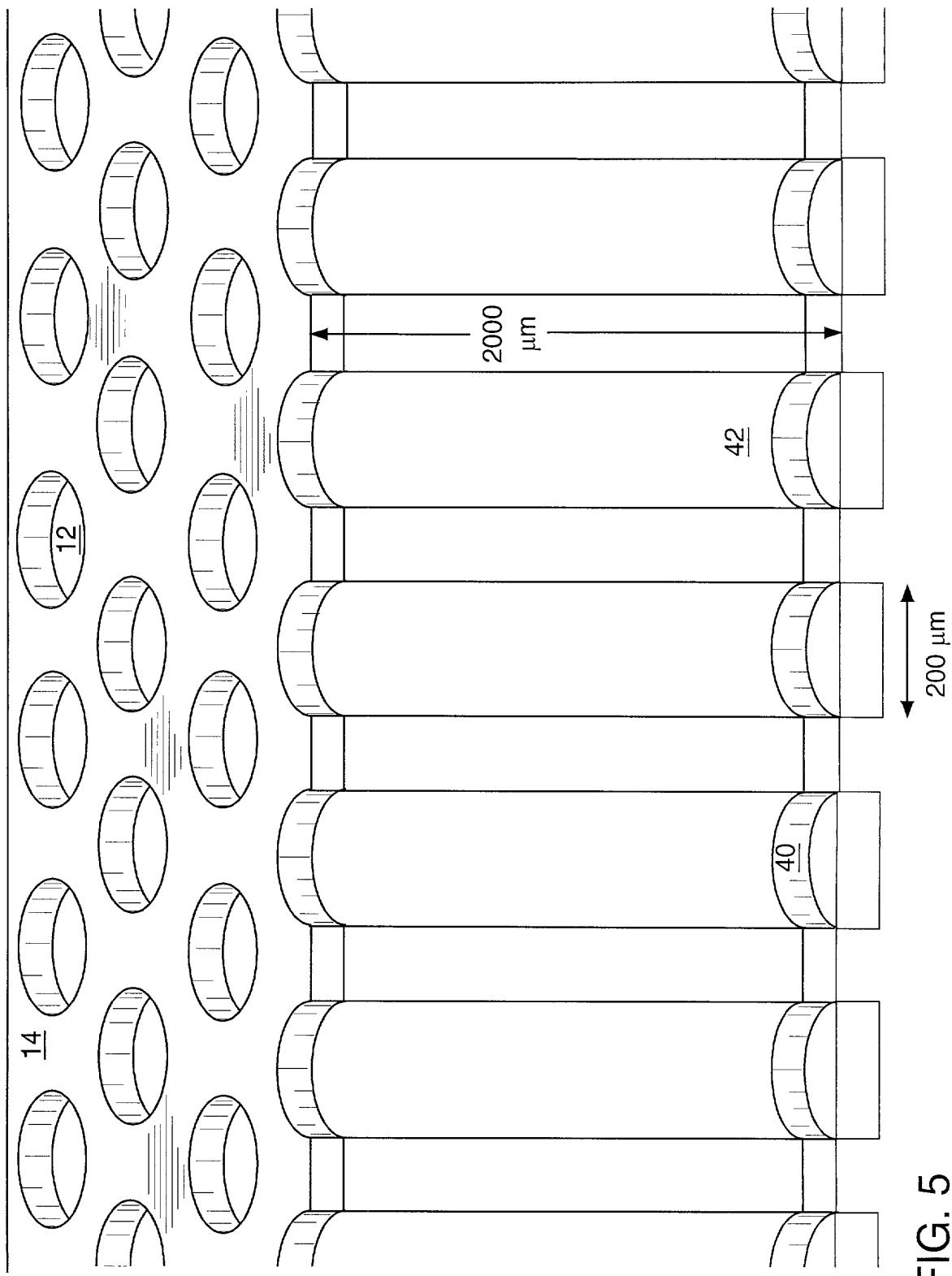


FIG. 5

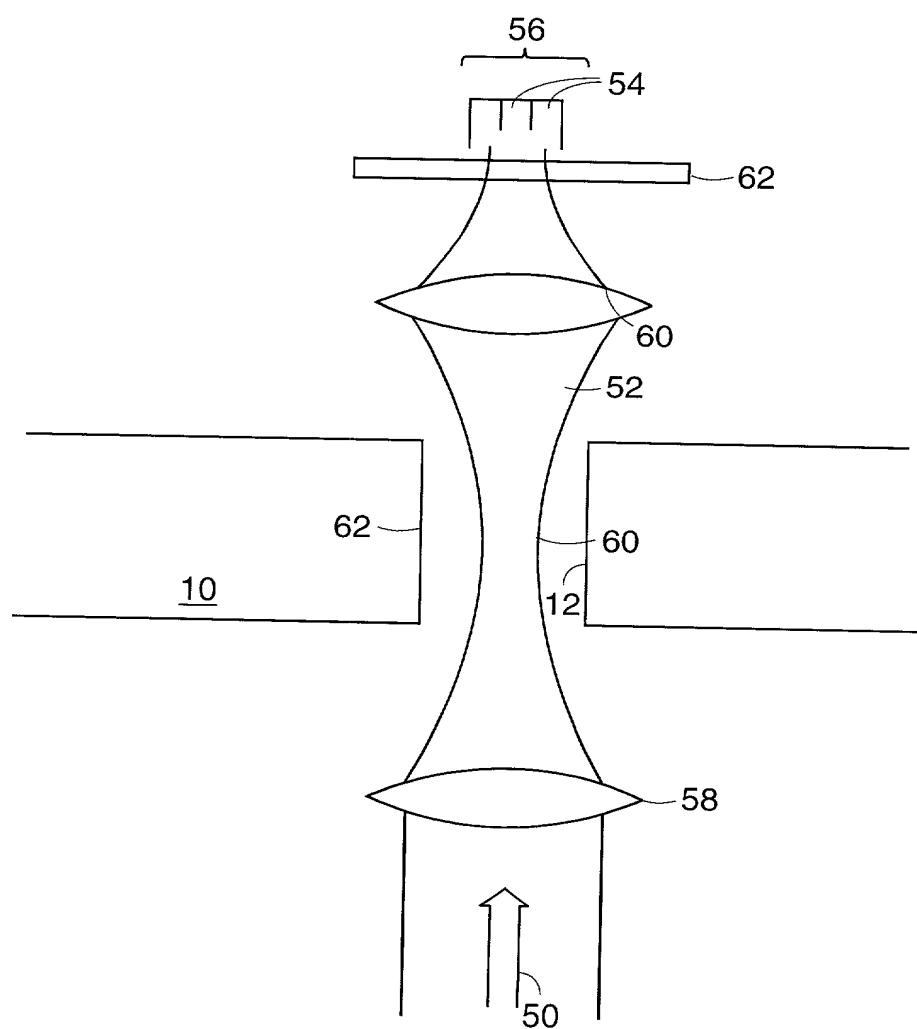


FIG. 6

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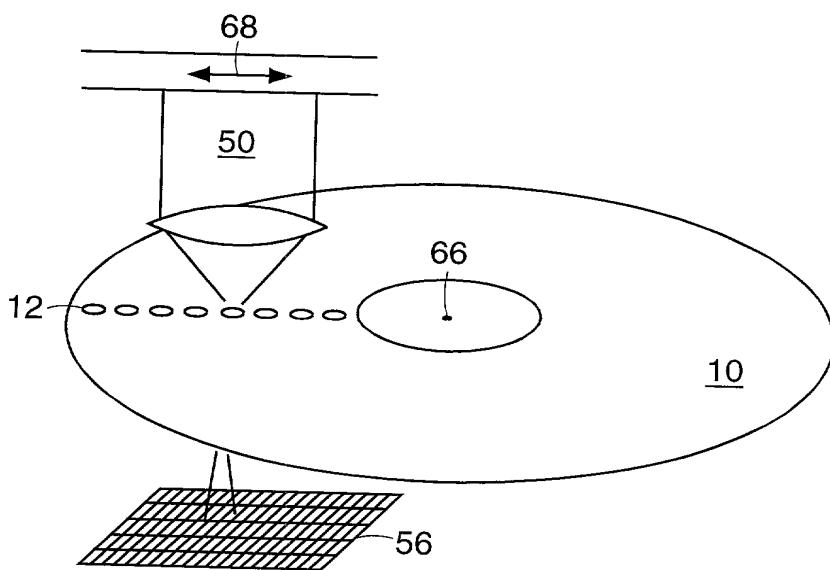


FIG. 7

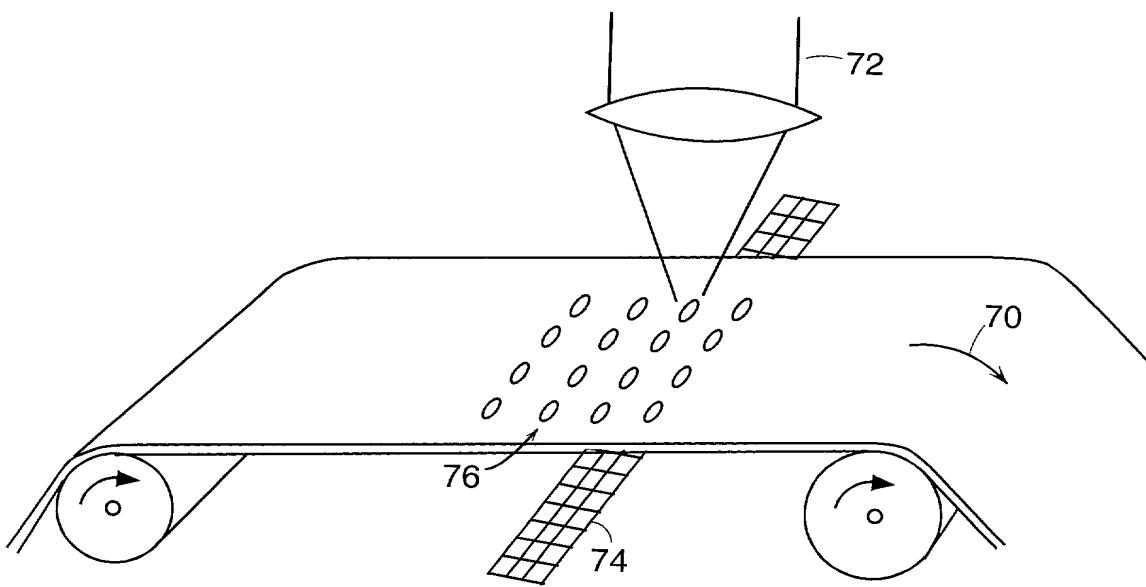
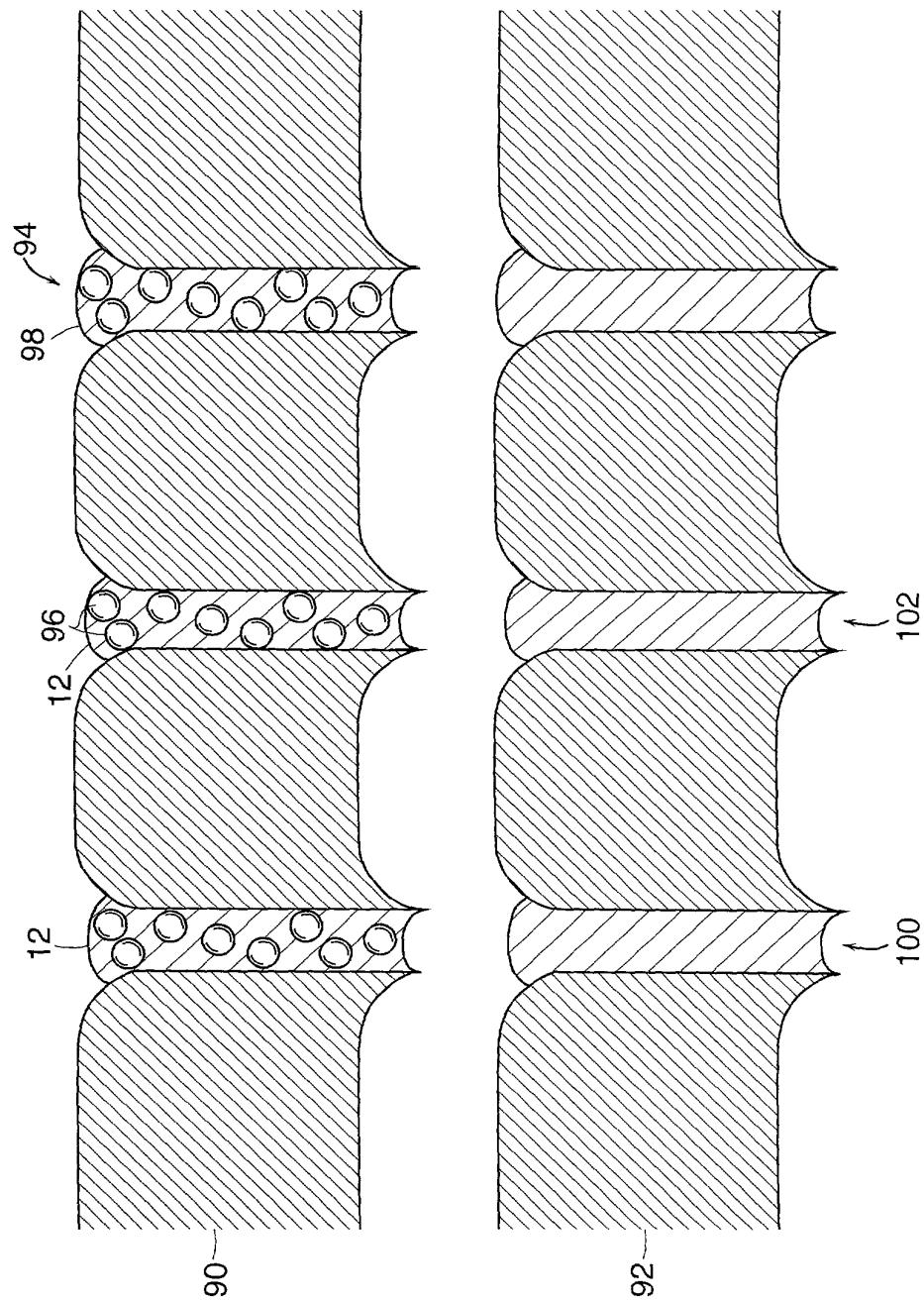


FIG. 8

FIG. 9



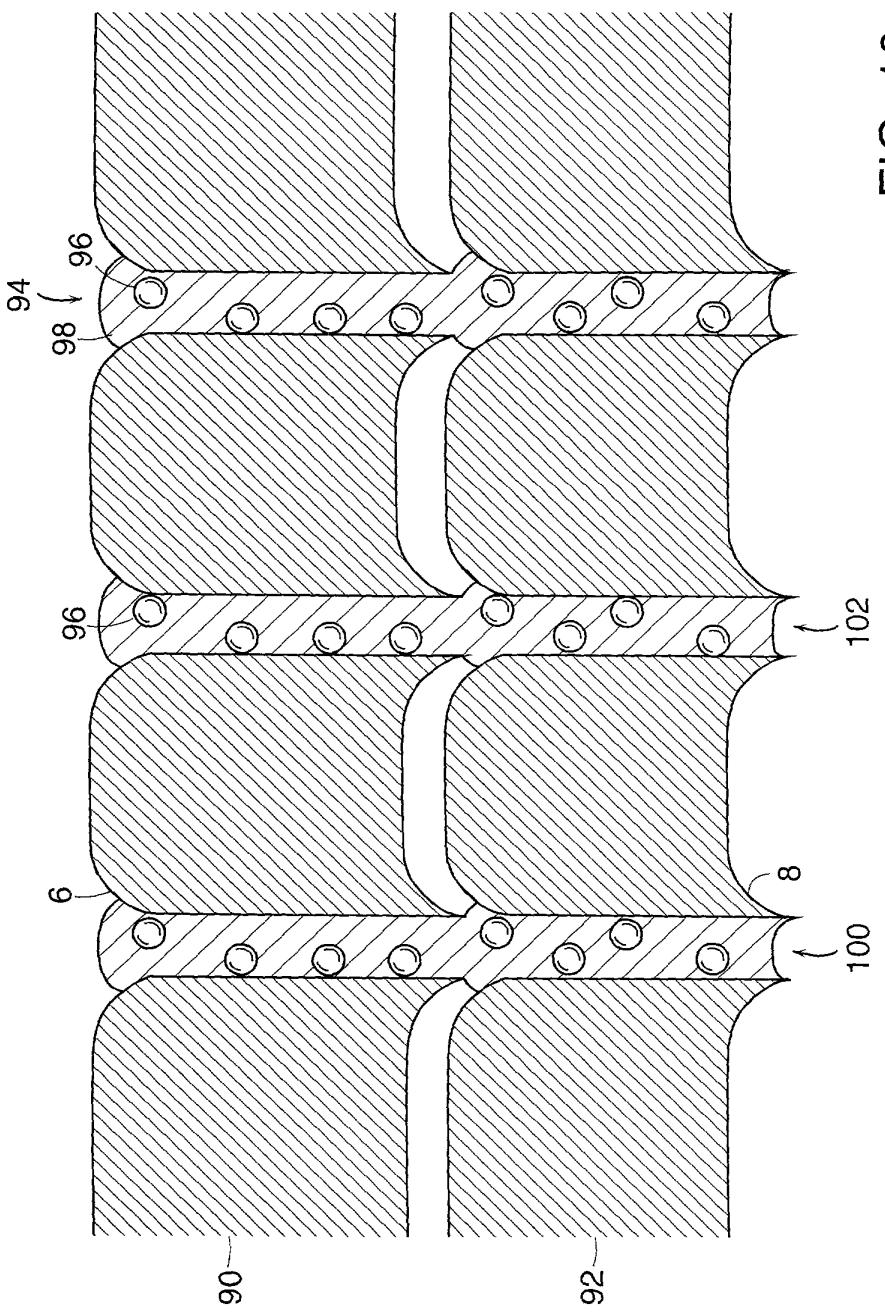


FIG. 10

Docket No.

1118/163

Declaration and Power of Attorney For Patent Application

English Language Declaration

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

METHOD AND APPARATUS FOR PERFORMING MICROASSAYS

the specification of which

(check one)

is attached hereto.

was filed on January 5, 1999 as United States Application No. or PCT International Application Number 09/225,583
and was amended on _____
(if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)

Priority Not Claimed

(Number)	(Country)	(Day/Month/Year Filed)	<input type="checkbox"/>
(Number)	(Country)	(Day/Month/Year Filed)	<input type="checkbox"/>
(Number)	(Country)	(Day/Month/Year Filed)	<input type="checkbox"/>

I hereby claim the benefit under 35 U.S.C. Section 119(e) of any United States provisional application(s) listed below:

<u>60/071,179</u>	<u>January 12, 1998</u>
(Application Serial No.)	(Filing Date)
(Application Serial No.)	(Filing Date)

I hereby claim the benefit under 35 U. S. C. Section 120 of any United States application(s), or Section 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. Section 112, I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, C. F. R., Section 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

<u>(Application Serial No.)</u>	<u>(Filing Date)</u>	<u>(Status)</u> (patented, pending, abandoned)
<u>(Application Serial No.)</u>	<u>(Filing Date)</u>	<u>(Status)</u> (patented, pending, abandoned)
<u>(Application Serial No.)</u>	<u>(Filing Date)</u>	<u>(Status)</u> (patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. (*list name and registration number*)

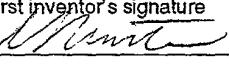
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